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Comparison of a modified assay method for the endopeptidase marker *Ep-D1b* with the Sequence Tag Site marker *XustSSR2001–7DL* for strawbreaker foot rot resistance in wheat

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Abstract

The endopeptidase marker *Ep-D1b* and Sequence Tag Site (STS) marker *XustSSR2001–7DL* were reported to be closely associated with the most effective resistance gene (*Pch1)* in wheat (*Triticum aestivum* L.) for strawbreaker foot rot [*Pseudocercosporella herpotrichoides* (Fron) Deighton]. Our objectives were to: (i) develop an efficient assay method for *Ep-D1b* in wheat; (ii) correlate endopeptidase zymograms to strawbreaker foot rot reactions of various wheat genotypes; and (iii) compare the utility of *Ep-D1b* and *XustSSR2001–7DL* for predicting disease response. An improved method of assaying for the *Ep-D1b* marker using roots from a single seedling was developed, which is a 2.5-fold improvement over the previous method. Thirty-eight wheat genotypes with known reactions to strawbreaker foot rot were analysed for *Ep-D1b* and the STS marker. Six distinct endopeptidase zymograms were identified among these 38 genotypes tested, and three of these patterns were novel. The endopeptidase marker was 100% accurate for predicting strawbreaker foot rot disease response, whereas the STS marker predicted the correct phenotype with approximately 90% accuracy. The endopeptidase marker *Ep-D1b* was more effective and was more economical for use in marker-assisted selection strategies for *Pch1* in our laboratory compared with the STS marker.

Key words: *Triticum aestivum* — eyespot disease — isozyme — marker-assisted selection — resistance gene

Strawbreaker foot rot (also known as eyespot), caused by *Pseudocercosporella herpotrichoides* (Fron) Deighton (teleomorph *Tapesia yallundae* Wallwork and Spooner) significantly reduces grain yields of wheat (*Triticum aestivum* L.) grown in North and South America, Europe, Australia, New Zealand and Africa (Wiese 1977, Fitt et al. 1988), and it is one of the most destructive winter wheat diseases in the Pacific Northwest (PNW) region of the US (Bruehl and Lai 1968, Murray 1983). Several resistance genes for strawbreaker foot rot have been reported, and the most effective gene, *Pch1*, is from *Aegilops ventricosa* (2n = 4x = 28, genomes $D^vD^vM^vM^v$), a wild relative of hexaploid wheat and is located on chromosome $7D^v$ (Sprague 1936, Jahier et al. 1978, Doussinault et al. 1983, Gale et al. 1984). *Pch1* was transferred to hexaploid wheat through cross-hybridization with repeated back crossing using the tetraploid wheat *T. turgidum* (AABB) as a 'bridge' species. Wheat cultivars, VPM1, TV1E-3H-9 and H-93–70, that were developed using this approach carry *Pch1* on a translocated segment on the distal end of the long arm of wheat chromosome 7D (7DL) (Kimber 1967, Maia 1967, Doussinault et al. 1983, Worland et al. 1988).

Endopeptidase-1 (Ep-1) in wheat is controlled by three loci, *Ep-A1*, *Ep-B1* and *Ep-D1* located on homoeologous chromosomes 7AL, 7BL and 7DL, respectively (Hart and Langston 1977, McMillin and Tuleen 1977, Koebner et al. 1988). *Ep-A1* has two alleles (*Ep-A1a* and *Ep-A1b*), *Ep-B1* has four alleles (*Ep-B1a*, *Ep-B1b*, *Ep-B1c* and *Ep-B1d*), and *Ep-D1* has two alleles (*Ep-D1a* and *Ep-D1b*; Koebner et al. 1988). The previous nomenclature for *Ep-D1a* and *Ep-D1b* was *EP-D1* and *EP-V1*, respectively. *Ep-D1a* is the endopeptidase allele from hexaploid wheat and *Ep-D1b* is the allele from *Ae. ventricosa* (McIntosh et al. 1995). Under the new nomenclature for wheat genes, *Ep-D1* refers to the locus and specific alleles are designated with letters. The *Pch1* and *Ep-D1* loci are both located on chromosome 7DL and McMillin et al. (1986) reported that the endopeptidase allele *Ep-D1a* in strawbreaker foot rot susceptible wheat cultivars was completely distinct from the endopeptidase allele *Ep-D1b* in *Ae. ventricosa* and in other resistant cultivars derived from *Ae. ventricosa*. The frequency of the *Ep-D1* alleles was compared with the strawbreaker foot rot reactions of a set of F_6 progenies from 30 $F_{2:5}$ genotypes derived from the cross VPM1/'Moisson 421'//N10/B-Sel101 (McMillin et al. 1986). An increased frequency of *Ep-D1a* was associated with susceptibility, whereas increased frequency of *Ep-D1b* was associated with resistance. Genotypes homozygous for *Ep-D1a* were least resistant, whereas genotypes homozygous for *Ep-D1b* had the highest level of resistance.

In the endopeptidase assay method of McMillin et al. (1986), 9-day-old etiolated shoots were homogenized for enzyme extraction using an ice-chilled mortar and pestle and crude extract was separated in a starch gel for 16–17 h. This procedure produced endopeptidase bands that were unclear and difficult to score consistently (D. E. Roberts, personal communication). The procedure was modified to use bulked roots from ten 5 to 6-day-old seedlings for enzyme extraction and 16 samples were loaded into preformed wells of starch gel resulting in clearer and more consistent bands (Roberts et al., unpublished). This procedure has been used for above 20 years in the USDA-ARS wheat breeding programme resulting in the development of several strawbreaker foot rot resistant cultivars including 'Hyak', the first wheat

cultivar developed using marker-assisted selection, and 'Madsen', 'Coda', 'Chukar' and 'Finch' (Allan et al. 1989, 1990, 2000, Garland Campbell et al. 2005a,b). Although the method was effective, it was slow and could not be used for early generation breeding materials as heterozygous individuals could not be distinguished from heterogeneous populations of individuals.

Groenewald et al. (2003) developed the STS marker *XustSSR2001–7DL* that was closely linked to the endopeptidase locus *Ep-D1*. The STS marker yielded a 222-bp band associated with *Ep-D1a* and a 240-bp band associated with *Ep-D1b*. Linkage between the *XustSSR2001–7DL* and the *Ep-D1* locus was determined based on segregation of marker alleles at both loci in two populations. Populations were derived from crosses between breeding lines homozygous for *Ep-D1a* and a 222-bp band and those homozygous for *Ep-D1b* derived from VPM1 and a 240-bp band. Two recombinants were detected among 98 progeny leading the authors to conclude that the STS marker was approximately 2 cM from the *Ep-D1* locus. It was, however, not reported if the STS marker is further from *Pch1* than *Ep-D1*. It was concluded that the 222-bp band is associated with susceptibility and the 240-bp band is associated with resistance to strawbreaker foot rot disease. Based on technical ease, the ability to assay a single seedling and the possibility of multiplexing with other PCR based markers it may be appealing to use the STS marker instead of the endopeptidase marker to select for *Pch1*. The utility of the STS marker for marker-assisted selection of strawbreaker foot rot resistance has not been previously reported.

The objectives of this study were to: (i) develop an efficient assay method for *Ep-D1b* in wheat; (ii) correlate endopeptidase zymograms to strawbreaker foot rot reactions of various wheat cultivars and breeding lines; and (iii) compare the utility of *Ep-D1b* and STS marker for predicting strawbreaker foot rot reaction.

Materials and Methods

Thirty-eight hexaploid cultivars and breeding lines of wheat, *Triticum aestivum* L., were obtained from the USDA-ARS wheat breeding programme, Pullman, WA, the Western Regional Uniform Cooperative Nurseries, and the Uniform Eastern Soft Winter Wheat Nursery (Table 1). As wheat is self-pollinated and all 38 genotypes were derived from pure lines, they were assumed to be homozygous. Reaction of these genotypes to strawbreaker foot rot was determined

Table 1: Thirty-eight wheat genotypes, sources or ID numbers, presence of *Pch1* in pedigree and strawbreaker foot rot reaction based on the genotyping data for the *Ep-D1b*, a linked STS marker and phenotypic evaluation in a strawbreaker foot rot disease screening nursery

Name of line	PI, ID number or source	Presence of *Pch1* in pedigree	Strawbreaker foot rot reaction based on		
			Ep-D1b marker	STS marker	Evaluation in field trials
VPM1	PI519303	Yes	Resistant	Resistant	Resistant
N10/B-Sel101	CItr13438	No	Susceptible	Susceptible	Susceptible
'Madsen'	PI 511673	Yes	Resistant	Resistant	Resistant
'Finch'	PI 628640	Yes	Resistant	Resistant	Resistant
'Chukar'	PI 628641	Yes	Resistant	Resistant	Resistant
'Coda'	PI 594372	Yes	Resistant	Susceptible	Resistant
ARS97119	USDA-ARS	Yes	Resistant	Resistant	Resistant
ARS97123	USDA-ARS	Yes	Resistant	Resistant	Resistant
ARS97134	USDA-ARS	Yes	Resistant	Susceptible	Resistant
ARS97135	USDA-ARS	Yes	Resistant	Resistant	Resistant
ARS9623	USDA-ARS	Yes	Resistant	Resistant	Resistant
99PHS-9595	USDA-ARS	No	Susceptible	Susceptible	Susceptible
99PHS-9604	USDA-ARS	No	Susceptible	Susceptible	Susceptible
99PHS-9607	USDA-ARS	No	Susceptible	Susceptible	Susceptible
99PHS-9610	USDA-ARS	No	Susceptible	Susceptible	Susceptible
99PHS-9621	USDA-ARS	No	Susceptible	Susceptible	Susceptible
99PHS-9637	USDA-ARS	No	Susceptible	Susceptible	Susceptible
99PHS-9641	USDA-ARS	No	Susceptible	Susceptible	Susceptible
99PHS-9645	USDA-ARS	No	Susceptible	Susceptible	Susceptible
99PHS-9648	USDA-ARS	No	Susceptible	Susceptible	Susceptible
'Brundage'	PI 599193	No	Susceptible	Susceptible	Susceptible
'Brundage96'	PI 631486	No	Susceptible	Susceptible	Susceptible
'Eltan'	PI 536994	No	Susceptible	Susceptible	Susceptible
WA7697	USDA-ARS	No	Susceptible	Susceptible	Susceptible
'Stephens'	CItr 17596	No	Susceptible	Susceptible	Susceptible
'Hill81'	CItr 17954	No	Susceptible	Susceptible	Susceptible
'Hiller'	PI 587026	No	Susceptible	Susceptible	Susceptible
'Zak'	PI 607839	No	Susceptible	Susceptible	Susceptible
'Lambert'	PI 583372	No	Susceptible	Susceptible	Susceptible
'Tubbs'	PI 629114	Yes	Resistant	Resistant	Resistant
P98134B3-1-4-6	Purdue Univ.	No	Susceptible	Resistant	Susceptible
P985RE1-16	Purdue Univ.	No	Susceptible	Susceptible	Susceptible
'Beamer'	WestBred L.L.C.	Yes	Resistant	Unpredictable	Resistant
'Mohler'	WestBred L.L.C.	Yes	Resistant	Resistant	Resistant
'West Bred 470'	PI 599335	No	Susceptible	Susceptible	Susceptible
'Semper'	CEBECO9303-A	Unknown	Susceptible	Susceptible	Susceptible
Residence	CEBECO9303	Unknown	Susceptible	Susceptible	Susceptible
Sunny	Sunbean Extract Co.	Unknown	Susceptible	Susceptible	Susceptible

in inoculated replicated field trials from 2000 to 2002 at the Spillman Research Farm in Pullman, WA, USA. No separate experiment was conducted for this data and it was obtained from the breeding programme. All genotypes were not evaluated each year for reaction to strawbreaker foot rot. The original culture of the strawbreaker foot rot pathogen was kindly provided by Dr Tim Murray, Department of Plant Pathology, Washington State University, Pullman, USA. Inoculation procedure was as described in Murray and Bruehl (1983). Disease was rated on a visual scale where 1 = no lodging with the absence of characteristic basal stem lesions and 5 = complete lodging with >85% of stems exhibiting lesions. Genotypes were considered resistant if averaged scores over three replications were two or lower and susceptible if averaged scores equalled or exceeded 4. An additional 511 experimental lines from the USDA-ARS wheat breeding programme were characterized for alleles at *Ep-D1* using the endopeptidase assay.

To harvest clean roots, wheat seeds were germinated in plastic growing trays (30 × 16 cm) constructed from a standard fluorescent light fixture grid trimmed to fit into a plastic box (30 × 18 × 8 cm; Rubbermaid Home Products Division, Fairlawn, OH, USA). Individual seeds were placed in grid sectors and the plastic box was filled with tap water until seeds were 50% submerged. This water level was maintained until roots were harvested. The seedlings were grown for 5–6 days in boxes on a laboratory bench at ambient temperature (20–25°C) and under the light of 60 W standard tungsten electric bulb with 16/8 h light/dark cycle.

The endopeptidase assay reported here was based on modifications of the method by McMillin et al. (1986). The procedures for preparation of the starch gel, electrophoresis tank and enzyme extraction buffers, slicing the gel after electrophoresis and the gel staining solution were described in detail in that report. The modifications were as follows: the starch gel was prepared using 18 g of hydrolyzed potato starch (Starch Art Corp., Smithville, TX, USA). The source of starch used in making starch gel is critical to the success of the assay (see discussion). Roots (approximately 4 cm) from a single 5 to 6-day-old seedling were excised and placed on a plastic weigh boat (2.5 × 2.5 cm) on ice. The enzyme was extracted by crushing the root tissue using a rectangular Plexiglas bar in 10 µl ice-cold extraction buffer. Two vertical slits, one approximately 3 cm and the other 9 cm from the cathode end of the gel, were cut across the starch gel. To load samples, Whatman No.3 filter paper strips (4 × 10 mm; Whatman Inc., Clifton, NJ, USA) were soaked in the enzyme extract for each genotype and inserted into the vertical slit with about 2 mm spacing between samples so that 20 samples were loaded in each slit of the gel resulting in 40 samples/gel. The gel was run in a 4°C refrigerator at 350 V and a constant current of 40 mA for 3–3.5 h. After electrophoresis, the gel slice was put in a plastic box (15 × 10 × 4 cm; Ain Plastics Inc, Mt Vernon, NY, USA) containing 25 ml of staining solution, sealed with a lid and then kept in dark at ambient temperature for 1–2 h. After staining, the gel was washed twice with deionized water and the gel was photographed using Foto/Analyst Mini Visionary^{TM} (Fotodyne Inc., Hartland, WI, USA).

Norin10/Brevor Selection101 (N10/B-Sel101; CItr 13438) and VPM1 (PI519303) are homozygous for *Ep-D1a* and *Ep-D1b*, respectively. The roots of N10/B-Sel101 and VPM1 were used, individually, or mixed together (roots from five plants each) to represent homozygous *Ep-D1a*, homozygous *Ep-D1b*, or heterozygous *Ep-D1a*/*Ep-D1b*, respectively. These three genotypes were assayed using the procedure of McMillin et al. (1986) with modifications (Roberts et al., unpublished) and with our modified procedure described above to verify that both methods produced identical results.

The 38 wheat genotypes were assayed for the endopeptidase using our modified method except for bulked roots from 10 seedlings were used for extraction as the genotypes were homozygous pure lines. Endopeptidase zymograms for the 38 genotypes were classified into the following categories: (i) Type-I if the banding pattern was identical to VPM1; (ii) Type-II if the banding pattern was identical to N10/B-Sel101; and (iii) Type-III if the banding pattern was identical to heterozygous or mixed class. Zymograms other than these three types were serially named as type-IV, V and VI. For the genotypes with type-III zymogram 20 individual seedlings were assayed for the endopeptidase in order to determine whether the pattern was due to heterozygosity at the endopeptidase locus, because of a heterogeneous mixture of genotypes, homozygous for *Ep-D1a* or *Ep-D1b*, or because of a unique banding pattern for a homogeneous, homozygous genotype. Each zymogram type was matched with strawbreaker foot rot reaction for each genotype to establish a relationship between the zymogram type and disease reaction.

The thirty-eight genotypes also were analysed for the STS marker *XustSSR2001-7DL* (Groenewald et al. 2003). Fresh leaf tissue bulked from the same 10 seedlings that had been used for the endopeptidase assay was used for isolating DNA following the CTAB extraction method of Anderson et al. (1992). The STS marker was assayed following the protocol of Groenewald et al. (2003) except that the forward primer was labelled with fluorescent label IRD-700 and the PCR amplified bands were analysed and visualized using 6.25% polyacrylamide gels on a Global IR^2 Analysis System (Li-Cor Biosciences, Lincoln, Nebraska). The strawbreaker foot rot reaction of each of the 38 genotypes was predicted as susceptible and resistant based on presence of the 222- and 240-bp band, respectively.

Results

Modification of the original protocol

The endopeptidase zymograms for both VPM1 and N10/B-Sel101 obtained from our modified protocol were identical to those obtained from the original protocol (Fig. 1a). VPM1 had the strawbreaker foot rot resistance specific top band for *Ep-D1b* whereas N10/B-Sel101 had the strawbreaker foot rot susceptibility specific bottom band for *Ep-D1a*. The middle band was present both in VPM1 and N10/B-Sel101. The mixture of VPM1 and N10/B-Sel101 had all three bands.

Endopeptidase zymograms and their association with the strawbreaker foot rot reaction

The endopeptidase zymograms observed among the 38 wheat genotypes (Fig. 1b) were grouped into six classes (I–VI) as described from the gel photographs along with the line diagrams of each type in Fig. 2 as follows: (i) The type-I zymogram had the top band for *Ep-D1b* and the middle band. (ii) The type-II zymogram had the middle band and the bottom band for *Ep-D1a*. (iii) The type-III zymogram had all three bands. The type-III zymogram could not be distinguished from the zymogram for heterozygous or heterogeneous loci (lane 3, Fig. 1). All 20 individual seedlings per genotype with the type-III zymogram produced the same result. (iv) The type-IV zymogram had only the top band for *Ep-D1b*. (v) The type-V zymogram had both the top band for *Ep-D1b* and the bottom band for *Ep-D1a*. (vi) In the type-VI zymogram, only the middle band was present. These six types were the only zymogram profiles observed among 511 additional experimental lines that were analyzed for the endopeptidase marker (data not shown). When the zymogram types of the 38 wheat genotypes were correlated with their strawbreaker foot rot reactions, all genotypes with type-I and type-IV were resistant to the strawbreaker foot rot and had *Pch1* gene in their pedigree, whereas genotypes with type-II, type-III, type-V and type-VI were susceptible and did not have *Pch1* gene in their pedigree (Fig. 2, Table 1). Therefore, the presence of top band for *Ep-D1b* along with the absence of bottom band for *Ep-D1a*

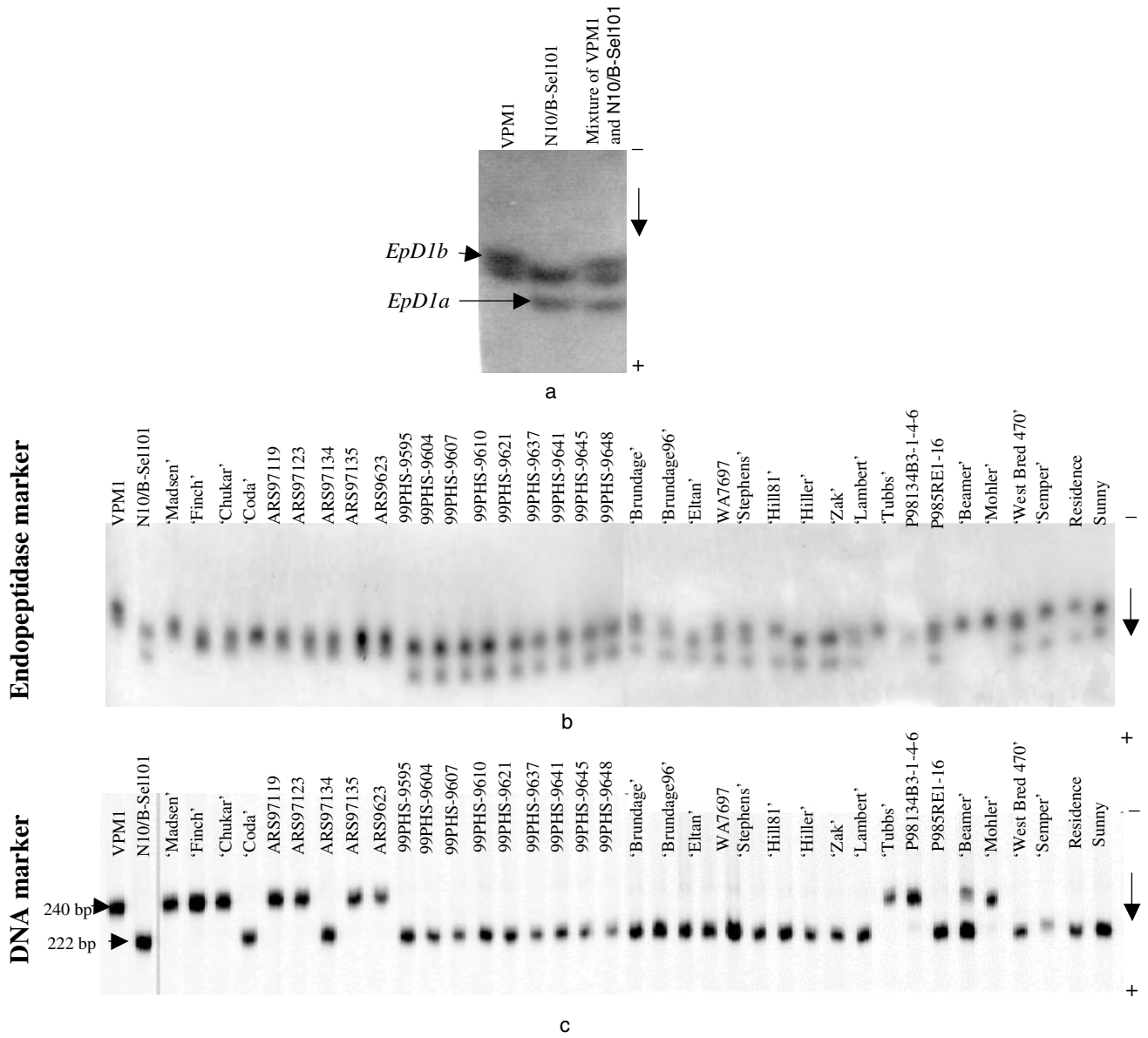

Fig. 1: Endopeptidase zymogram, STS-marker profile of wheat genotypes differing in strawbreaker foot rot reaction. (a) Typical zymograms of endopeptidase in resistant genotype VPM1 (homozygous for *Ep-D1b*), susceptible genotype N10/B-Sel101 (homozygous for *Ep-D1a*), and mixed VPM1 and N10/B-Sel101 (five seedlings each) to represent heterozygous *Ep-D1a*/*Ep-D1b*; (b) Endopeptidase zymograms of the 38 wheat genotypes; and (c) Amplified products for the STS marker *XustSSR2001-7DL* for the 38 wheat genotypes. Amplified bands 222 bp and 240 bp indicate strawbreaker foot rot susceptibility and resistance specific bands, respectively

in a genotype is associated with resistance to strawbreaker foot rot. In contrast, either the presence of bottom band for *Ep-D1a* regardless of the presence or absence of top band for *Ep-D1b* or the absence of both bottom and top bands for *Ep-D1a* and *Ep-D1b*, respectively as in cultivar P98134B3-1-4-6 is associated with susceptibility to strawbreaker foot rot.

The STS marker genotype and its association with the strawbreaker foot rot reaction

The STS marker profiles of the 38 genotypes were analysed for the presence of either a 222- or 240-bp band (Fig. 1c). The STS marker amplified the 240-bp band in 10 genotypes, resistant to strawbreaker foot rot and had *Pch1* in their pedigree. Twenty-four genotypes had the 222-bp band, were susceptible to the disease and did not have the *Pch1* in their pedigree. Two genotypes had the 222-bp band but were resistant to the disease and had *Pch1* in their pedigree. The breeding line P98134B3-1-4-6, which did not have *Pch1* in its pedigree had the 240-bp band but was susceptible, whereas the cultivar 'Beamer' that had *Pch1* in its pedigree had both the 240- and 222-bp bands and was resistant. In order to determine if the presence of both bands in 'Beamer' was because of heterozygosity at the STS marker locus or because of a heterogeneous mixture of genotypes, homozygous for 222- or 240-bp band, 48 single seedlings were assayed for the STS marker. The 222-bp band was present in 32 individual seedlings, whereas 16 seedlings had the 240-bp band. Reaction to strawbreaker foot rot of these individual seedlings with 222- and 240-bp band was not evaluated.

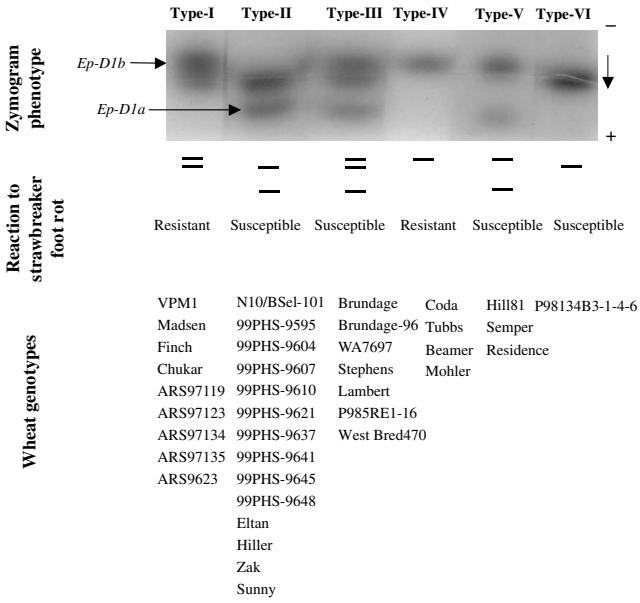

Fig. 2: Endopeptidase zymogram types and their relationship with strawbreaker foot rot reaction. Wheat genotypes representing the zymogram type are located below the lane for that type

The endopeptidase zymogram based prediction of strawbreaker foot rot reaction for the 38 wheat genotypes was compared with the STS-marker-based prediction (Table 1). The endopeptidase based prediction of the disease reactions of all 38 genotypes was in complete agreement with the reaction to the disease obtained from the field evaluation. The predicted disease reaction based on the STS-marker genotype was correct in 34 of the 37 genotypes (Table 1). Disease reaction of 'Beamer' could not be predicted based on the STS marker.

Discussion

The advantages of using the modified protocol for the endopeptidase assay include: (i) detection of heterozygosity and heterogeneity is possible because tissue can be excised from a single seedling; and (ii) throughput is increased by loading 40 samples in a single starch gel.

The source of hydrolyzed potato starch used in making starch gel is critical for the success of the assay. When the gel was prepared using the hydrolyzed potato starch (catalogue no. S-5651) bought from Sigma (St. Louis, MO, USA), the gel could not be sliced into single piece for staining. However, there was no problem in slicing when the gel was prepared using hydrolyzed potato starch bought from Starch Art Corp. (Smithville, TX, USA) was used.

Among the six zymogram types reported here, the type-I, type-II and type-III were observed in previous reports (McMillin et al. 1986); and type-IV, type-V and type-VI are novel. The type-III zymogram observed by McMillin et al. (1986) in F_6 progenies from $F_{2:5}$ genotypes was because of a heterogeneous mixture of genotypes, whereas the type-III zymogram observed in genotypes derived from pure lines in this report is unique and not because of heterogeneity or heterozygosity as all 20 individual seedlings per genotype with the type-III zymogram produced the same result (data not shown).

Comparisons of the endopeptidase zymograms and strawbreaker foot rot reactions indicated that the presence of the bottom band for *Ep-D1a* was associated with disease susceptibility regardless of the presence or absence of the top band for *Ep-D1b*. The bottom band for *Ep-D1a* appeared to be dominant to the top band for *Ep-D1b*, although reaction to strawbreaker foot rot was not due to the products of the *Ep-D1a* or *Ep-D1b* (Mena et al. 1992). The middle band was not correlated with the disease reaction. This band has been reported to be a mixture of the co-migrating products from *Ep-A1* and *Ep-B1* loci (Hart and Langston 1977), which is consistent with the fact that the loci *Ep-A1* and *Ep-B1* are mapped to chromosome 7AL and 7BL, respectively (Koebner et al. 1988) and *Pch1* is located on chromosome 7DL. The genotype P98134B3-1-4-6 (zymogram type-VI) did not have either the bottom band for *Ep-D1a* or the top band for *Ep-D1b*. This cultivar carries the barley yellow dwarf mosaic virus resistance gene *Bdv2* on the distal end of chromosome 7DL translocated from the wild wheat-grass *Thinopyrum intermedium* (Brettell et al. 1988, Banks et al. 1995, Hohmann et al. 1996, Wang and Zhang 1996). Therefore, we assume that the absence of both *Ep-D1a* and the *Ep-D1b* alleles in P98134B3-1-4-6 may be because of the fact that the new region does not have the *Ep-D1* or has novel bands that are not detected because of translocation.

Results in this report documented that the endopeptidase marker is 100% accurate for predicting strawbreaker foot rot reaction, whereas the STS marker predicted disease reaction with approximately 90% accuracy. Endopeptidase marker based prediction of strawbreaker foot rot reaction also matched with the presence or absence of the *Pch1* gene in pedigrees of the 34 genotypes for which pedigrees were known. STS marker based prediction of strawbreaker foot rot reaction was incorrect in 'Coda', ARS97134 and P98134B3-1-4-6. The cultivar 'Beamer' was a heterogeneous mixture of genotypes at the STS marker locus; however, it was homogeneous at the *Ep-D1* locus. We considered labour and chemical cost to calculate price of each data point for endopeptidase and STS markers. The endopeptidase marker was found to be efficient and was more economical than the STS marker in our laboratory. The number of samples evaluated per starch gel can be further increased by using larger gel rigs. The STS marker assay may be more efficient and more economical when it is multiplexed with other DNA markers, but care should be taken when using the STS marker for marker-assisted selection of *Pch1* because approximately 10% of genotypes might be inaccurately classified as resistant to strawbreaker foot rot based on the presence of the 240-bp band. Use of the STS marker would be improved if a flanking marker distal to *Pch1* could be identified. We concluded based on this report that endopeptidase marker *Ep-D1b* is more efficient and effective in marker assisted selection for strawbreaker foot rot resistance than the STS marker *XustSSR2001-7DL*.

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